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Evaluation of an Analytical Method for an in-vitro Study of Degradation of Organochlorine Compounds by 'Meat Starter' Micro-organisms*

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Abstract: The method of analysis employed in an in-vitro study on degradation of organochlorine compounds by meat starter micro-organisms was evaluated using the recommendations made by the Food and Drug Administration (FDA).

Recoveries of the organochlorine pesticides α-, β- and γ-hexachlorocyclohexane, hexachlorobenzene, *pp*′DDE, dieldrin and the polychlorinated biphenyl PCB 153 from a mixture added to four different liquid media, tryptic soy broth, brain heart infusion, a commercial broth and a mineral salts medium, were determined. Recoveries were between 80 and 110% with all components, irrespective of the medium used, and there were no analytical interferences due to the reagent blank or to the matrix. The repeatability was very good with relative standard deviations in the range 2·5 to 11·1%. The concentration of each component of the mixture was determined by capillary GC analysis of extracts using an electron capture detector.

Key words: organochlorine and polychlorobiphenyl compounds, GC analysis, EC detector

1 INTRODUCTION

The widespread presence of residues of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in food has arisen from their extensive agricultural application and industrial emission in the environment.

One of the major problems in the use of such chemicals is their persistence in nature. Microbial degradation of organochlorine pesticides and polychlorinated biphenyls could be an important route of degradation of these widespread pollutants. Nowadays, in the meat industry it is very common to use microbial starter cultures to improve the characteristics of the meat products. Residues of OCPs and PCBs have been found in meat products and the possibility that these micro-organisms could degrade these contaminants is of great interest. In order to investigate such a possibility, some in-vitro studies on the degradative activity of microbial starter cultures from the meat industry were performed in our laboratory.[1] Capillary gas-liquid chromatography with electron-capture detector (GLC-ECD) was used to investigate and quantify the transformation of such residues.

The accurate quantification of the contaminant that is degraded by the micro-organisms is of great importance, and is related to the analytical method developed. The following minimum evidence is required to demonstrate that a method is valid for a particular analyte in a given matrix.[2]

(1) A reagent blank analysis performed using reagents only (no sample) shows no detector responses that could be mistaken for that of the analyte.
(2) Concurrent analyses of a residue-free lot of the same or similar matrix show no interfering detector responses.

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TABLE 1
Experimental Chromatographic Conditions with the 007-2 Column

Parameter	
Column length (m)	50
Column internal diameter (mm)	0·25
Film thickness (μm)	0·25
Automatic injection	Yes
Injector splitless	Yes
Injection volume (μl)	2
Injector temperature (°C)	210
Detection	^{63}Ni ECD
Detector temperature (°C)	300
Carrier gas	N_2
Linear carrier gas velocity (cm s^{-1})	33·5
Make-up gas	N_2
Make-up gas flow-rate (ml min^{-1})	55
Initial oven temperature (°C)	125
Initial isothermal period (min)	2
Initial programming rate (°C min^{-1})	10
Second isothermal temperature (°C)	204
Second programming rate (°C min^{-1})	2
Final isothermal temperature (°C)	290
Final isothermal period (min)	11

(3) Recovery of the analyte added to a residue-free sample is in the range 80 to 110%.

The accuracy of analytical methods for residues is commonly evaluated in terms of percentage recovery of each component added to a particular sample. The basic requirement is to estimate how much of the analyte has been removed from the natural matrix by a given extraction technique. To determine the efficiency of extraction, the widespread practice is to add a known amount of the analyte to the matrix, usually in an organic solvent, prior to extraction and subsequent analysis. This type of spiked sample analysis will determine the accuracy and precision of the subsequent analytical steps, but does not necessarily measure the efficiency of extraction, because for that, it is imperative that the contaminant is bound to the matrix in a similar manner to that which exists in the environment. At present, water is the only matrix where this can be achieved in a relatively straightforward way. The analytes are added below the surface of the sample in a small quantity of a water-miscible solvent (e.g. acetone or methanol).[3]

All measurements are subject to variability. As soon as a second measurement of any continuous property is taken, a high probability exists that this value will not be the same as the first.[4] Repeatability is the closeness of agreement between successive results obtained with the same method on identical test material, under the same conditions (performance by the same operator, with the same apparatus, in the same laboratory at the same moment or with a short interval only).[5] The relative standard deviation (RSD) is the most useful measure of precision in chemical analytical work, because the RSDs are usually independent of concentration or amount of analyte over a reasonable range of concentrations.[6]

The sensitivity of electron capture detectors (ECD) is such that care must be taken to ensure that no interference occurs from samples, reagents, media, etc. Thus, a procedural blank consisting of all reagents and glassware used during analysis must be performed to check for interferences and cross-contamination.

The aim of this study was to evaluate the method used for the analysis of the following compounds: α-, β- and γ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), *pp*′DDE, dieldrin and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), added to the liquid media tryptic soy broth (TSB), mineral salt medium (MSM), a commercial broth (*Lactobacillus* broth for microbiology according to De Man, Rogosa and Sharpe, MRS) and brain heart infusion (BHI) to be used in the in-vitro study of degradation of organochlorine contaminants by meat starter micro-organisms.

2 MATERIALS AND METHODS

2.1 Chemicals

HPLC grade acetone and hexane, and iso-octane of pesticide grade (Pestiscan) were purchased from Lab-Scan. The purity of the solvents was determined by capillary gas-liquid chromatography with electron-capture detection (GLC-ECD) in the form in which they would be used in the experimental procedure. Acetone and iso-octane were analysed directly and the purity of hexane was determined after evaporation of a 60-ml sample of solvent to 10 ml.

Sodium sulfate (anhydrous) was obtained from Carlo Erba and it was purified by heating in a furnace at 600°C for 5 h, the cooled product was used for drying organic extracts.

Standard samples of α-HCH (99·6% purity), β-HCH (99·1% purity), HCB (99·6% purity), *pp*′DDE (99·7% purity), dieldrin (98·1% purity) and PCB 153 (99·1% purity) were supplied by Laboratory Dr Ehrestorfer; that of γ-HCH (100% purity) was from Supelco.

Water was distilled using a MilliQ water purification system and extracted with hexane prior to preparation of the liquid media.

The liquid media TSB and BHI were from Difco, MRS was from Merck, and the mineral salts medium contained (g $litre^{-1}$): Na_2HPO_4 (7); KH_2PO_4 (3); NaCl (0·5); NH_4Cl (1); $MgSO_4.7H_2O$ (0·25) and yeast extract (Difco) (1).

All glassware was cleaned with water and soap, rinsed with distilled water and dried. It was rinsed with organic solvent prior to use.

TABLE 2
Recoveries of Diverse Organochlorine Pesticides and the Polychlorinated Biphenyl PCB 153 from Different Liquid Media

	Recovery (%)[a]							
	TSB[b]		MSM[c]		MRS[d]		BHI[e]	
Compound	Mean	RSDr[f]	Mean	RSDr[f] *a*	Mean	RSDr[f]	Mean	RSDr[f]
HCB	85·2	3·3	87·4	4·5	86·3	4·5	92·4	11·1
α-HCH	85·1	3·4	88·0	4·5	93·5	5·2	92·9	10·5
β-HCH	84·0	2·5	88·1	4·3	93·0	6·4	93·0	7·8
γ-HCH	86·4	3·3	89·8	4·0	93·8	5·2	95·9	9·4
pp′DDE	84·1	3·8	88·6	4·8	85·0	6·3	88·0	7·9
Dieldrin	85·2	3·2	89·4	4·7	91·0	5·4	93·1	8·4
PCB 153	82·1	4·2	85·8	4·7	82·8	5·4	87·7	7·6

[a] Results are mean of eight assays. In each, 9·9 ml of the liquid medium was spiked with 0·1 ml of a standard solution made up as a OCP/PCB mixture in acetone in which each compound was present at 100 μg ml^{-1}.
[b] Tryptic soy broth.
[c] Mineral salt medium.
[d] De Man Rogosa and Sharpe medium.
[e] Brain heart infusion.
[f] Repeatability relative standard deviation.

2.2 Apparatus

The extractions were carried out using an automatic side-arm shaker (Vibromatic, P. Selecta).

Chromatographic analyses were performed with a Hewlet-Packard HP 5890 system with ^{63}Ni electron capture detector (ECD), equipped with an automatic injector HP 7673A. A fused silica capillary column coated with 5% phenyl/95% methyl polysiloxane (007-2, 50 m × 0·25 mm I.D. × 0·25 μm film thickness) was used for determination. Data acquisition and processing were performed on a HP Vectra 486/33U computer using Hewlett-Packard Chemstation software. Eight replicate analyses were performed in each case and operating conditions for GC-ECD are in Table 1.

2.3 Sample treatment

Tubes containing 9·9 ml of the sterilised liquid medium were spiked with 0·1 ml of a standard solution made up as a OCP/PCB mixture in acetone in which each compound was present at 100 μg ml^{-1}. Eight replicates of each spiked medium were prepared. For blank analyses, tubes containing 9·9 ml of each sterilised liquid medium were inoculated with 0·1 ml of acetone.

2.4 Extraction technique

This followed the general method described by Viney and Bewley[7] with slight modifications. The medium (10 ml), either blank or spiked with the OCP/PCB mixture, was shaken with hexane (40 ml; 1 h) and the liquid then transferred to a separatory funnel, using hexane (5 ml) as a rinse. The organic layer was removed, rinsing the separatory funnel with hexane (5–15 ml), dried with sodium sulfate (only in the case of MRS and BHI media) and the volume reduced to 10 ml in a rotary evaporator before it was transferred to a vial. This solution was diluted 10-fold with hexane before analysis.

To check for interferences due to the reagents or the matrix, blank analyses were performed with each medium.

2.5 Recovery

Recovery of the OCPs and PCB 153 was calculated by comparing the chromatograms of each assay with the chromatogram of a standard solution prepared by adding 0·1 ml of the mixture of the organochlorine compounds in acetone (in which each compound was present at 100 μg ml^{-1}) to 9·9 ml of iso-octane, and 10-fold diluted.

The descriptive statistical analysis of the results was made with the use of Statview SE + Graphics™ (Abacus Concepts, Inc., 1988, Berkeley, CA) for personal computers.

3 RESULTS AND DISCUSSION

Table 2 shows the recoveries of each residue in the different liquid media. Percentages of recovery for all residues in the different matrices were in the range

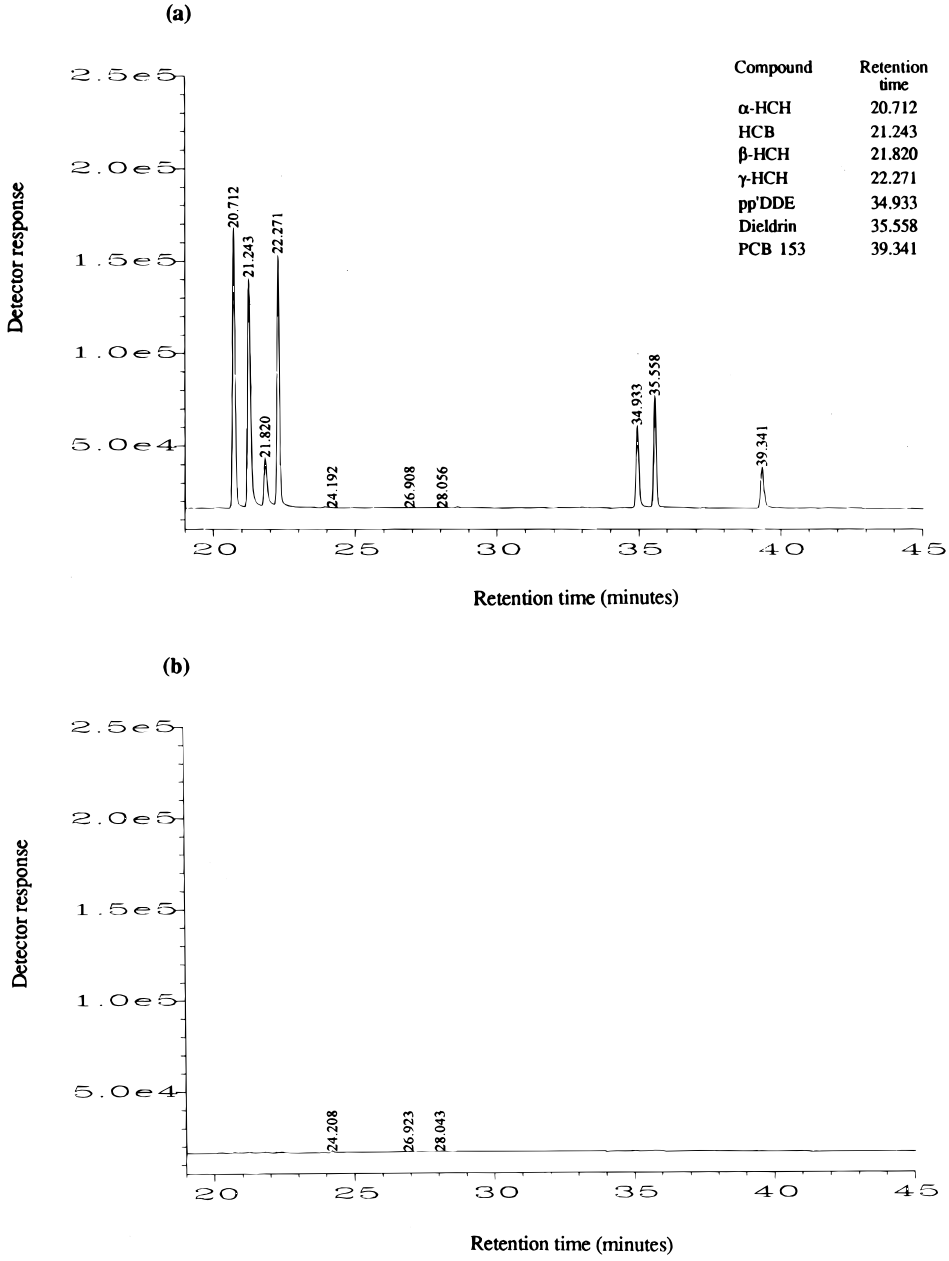

Fig. 1. Chromatograms of the extracts of the different liquid media spiked with the organochlorine/polychlorinated biphenyl mixture compared with those for the media from which the samples were extracted (GC-ECD using a 007-2 column, 50 m × 0·25 m ID; film thickness 0·25 m; injection volume 2 μl). (a), (b) TSB medium; (c), (d) MS medium; (e), (f) MRS medium; (g), (h) BHI medium.

80–110% as recommended by the FDA.[2] The repeatability was very good with low relative standard deviations (2·5–4·2% in TSB, 4·0–4·8% in MSM, 4·5–6·4 in MRS and 7·6–11·1% in BHI). Analyses of blanks showed that there were no interfering substances which co-eluted with an analyte in any case (Fig. 1(a), (b); 1(c), (d); 1(e), (f) and 1(g), (h).

Smart,[8] after reviewing all published and some unpublished collaborative studies of methods for pesticide residues, suggests that the limits of acceptability of a method require that spiked recoveries lie between 70 and 110% with a mean above 80%; the coefficient of variation (C.V.) should usually be within the range of 5 to 15%, but when it is above 20%, the method exhibits too much variability. Our results agree with the statement of Smart[8] because the mean recovery of all compounds was >80%, and the variation (RSD) was below 15% in all cases.

Hess *et al.*[3] stated that it is difficult to be categorical about the percentage level of recovery which is regarded as acceptable for a method. Some workers accept values of <60%. Where methods give a recovery of <75% it

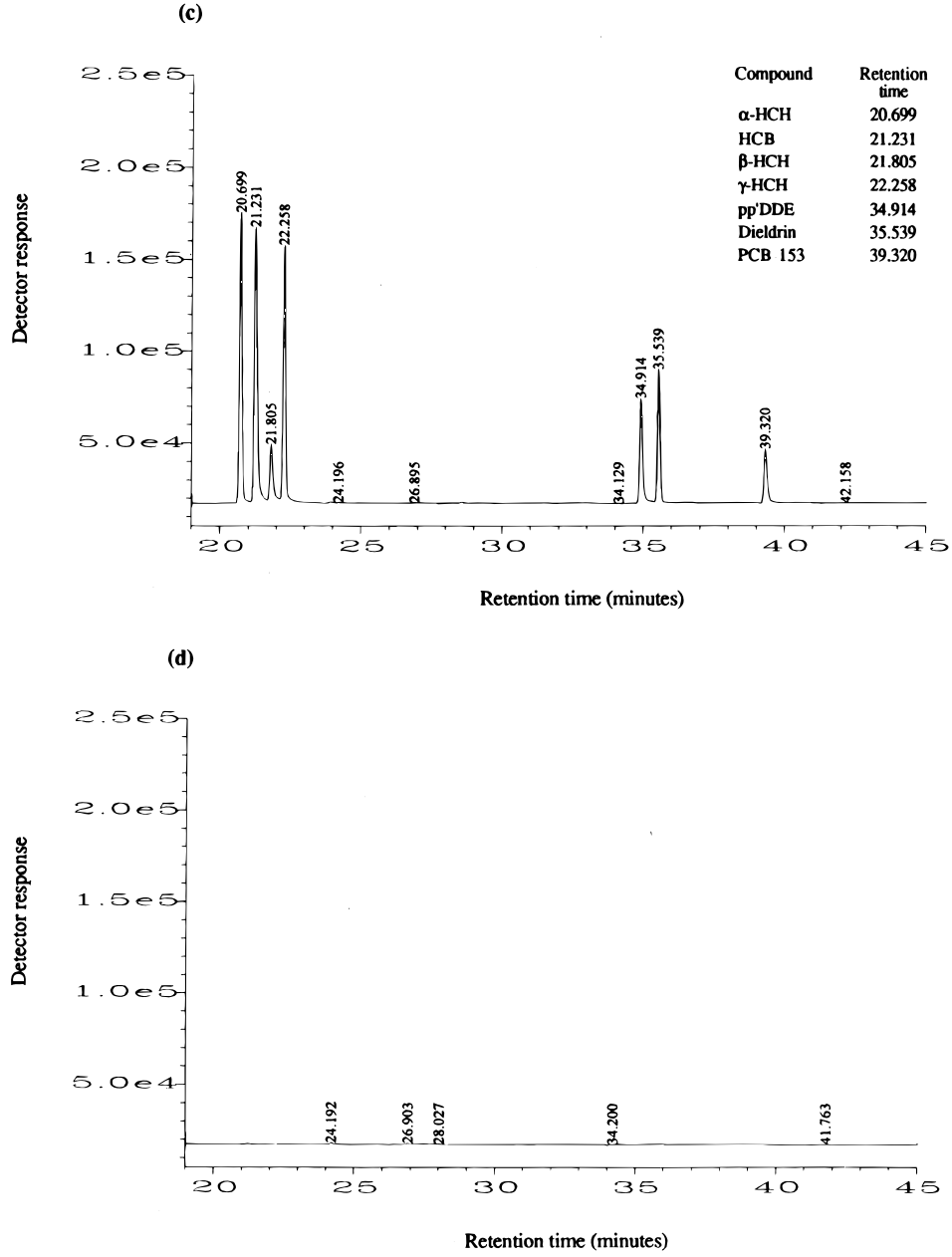

Fig. 1. continued

is essential to determine whether this low value is dependent on the specific type of matrix structure. Methods with such a low recovery are also likely to have a greater variance associated with the precision of the measurement. In the present study, the mean recovery of all compounds was well above 75% and the results demonstrated good repeatability.

It is difficult to compare our results with the results of other authors, because they used different culture media from ours, and different analytical methodology. Moreover, publications of recovery and repeatability of the analytical method for this type of *in-vitro* study are scarce. The results of Katayama and Matsumura[9] were higher than our results for DDT and dieldrin, ranging between 92(±5)% and 95(±6)%. Sahu *et al.*[10] using hexane for extraction, recovered more than 95% of the three isomers of HCH (α-, β- and γ-) from deionised water. Similarly to us, these authors recovered between 80 and 110% of the investigated residues.

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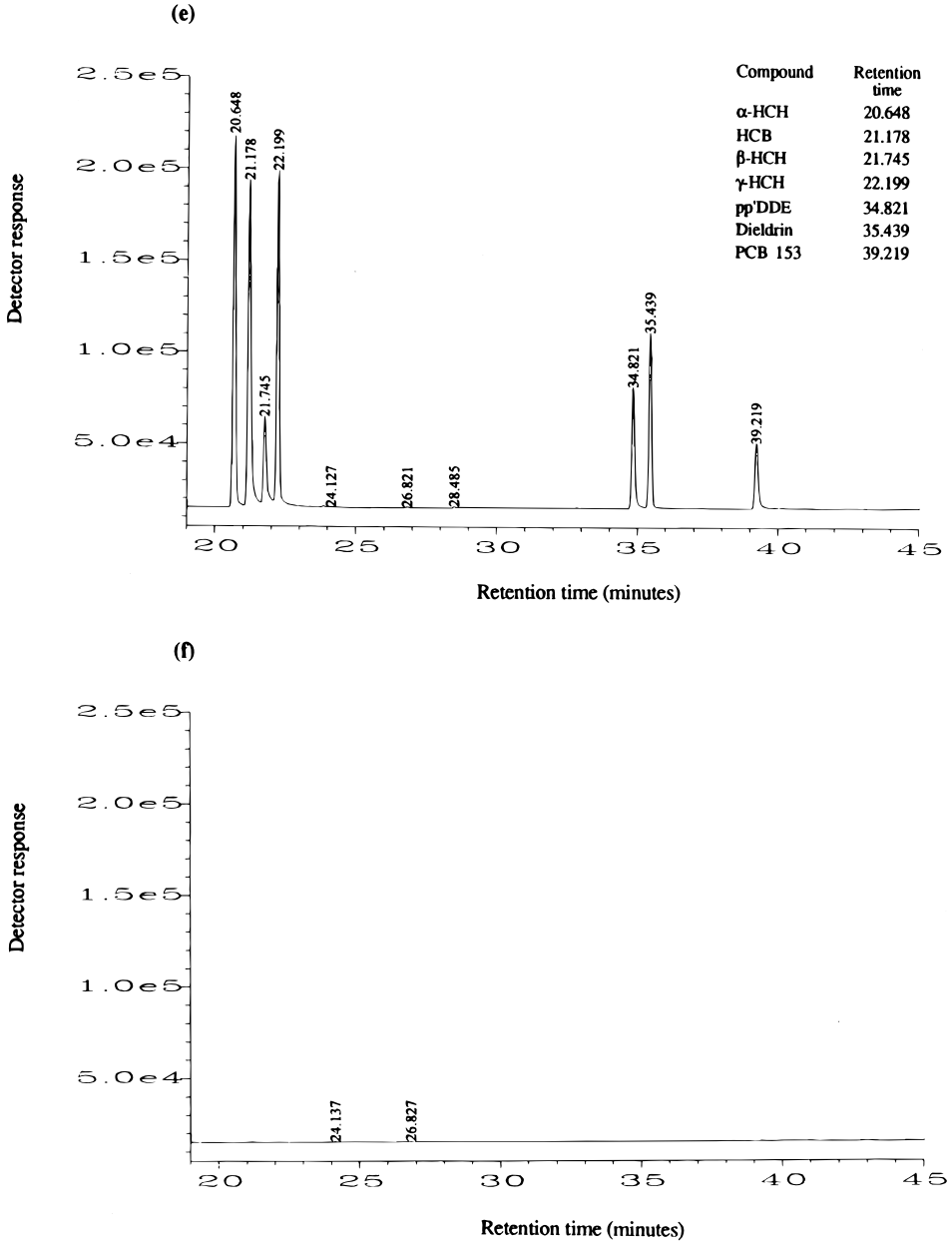

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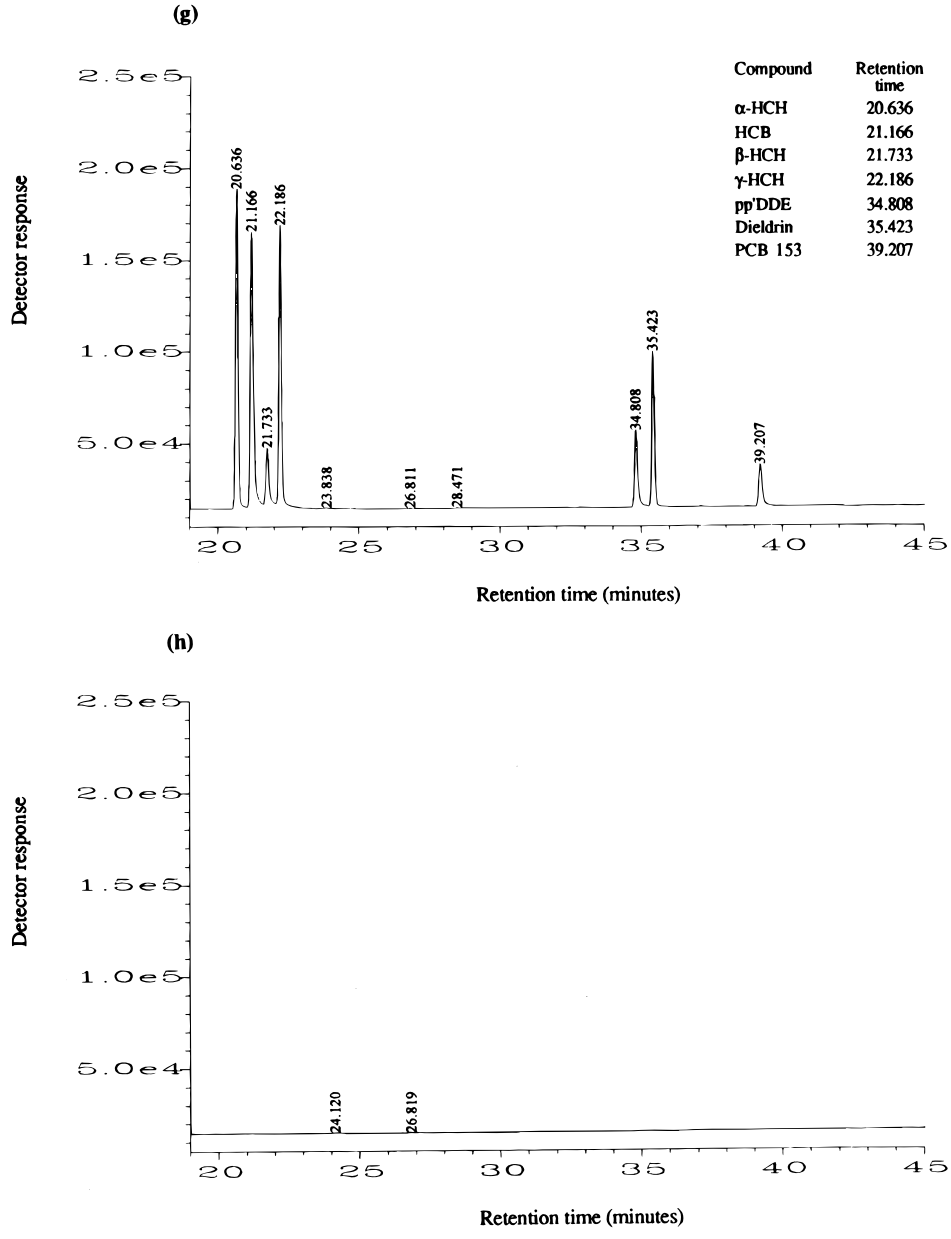

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REFERENCES

1. Bayarri, S., Herrera, A., Ariño, A. A., Lázaro, R. & Conchello, P., A study of the degradation *in vitro* of organochlorine compounds by the meat starter microorganism *Micrococcus varians*. *Pestic. Sci.* **50** (1997) 147–51.
2. Food and Drug Administration (FDA) *Pesticide Analytical Manual. Volume I. Methods which detect multiple residues.* 3rd edn. US Department of Health and Human Services, 1994.
3. Hess, P., de Boer, J., Cofino, W. P., Leonards, P. E. G. & Wells, D. E., Critical review of the analysis of non- and mono-ortho-chlorobiphenyls. Review. *J. Chromatog. A*, **703** (1995) 417–65.
4. Horwitz, W., The inevitability of variability in pesticide residue analysis. *Adv. Pestic. Sci.*, Part 3 (1979) 649–55.
5. Haagsma, N., Ellen, G., Stephany, R. W. & de Ruig, W. G., Definitions for the determination of drug residues in food of animal origin (Discussion Group for Residue Analysis, ORA, Report 1995).
6. Helrich, K. (ed.), *Official methods of analysis.* Vol I, 15th edn. Association of Official Analytical Chemists (AOAC). Virginia, USA, 1990.
7. Viney, I. & Bewley, R. J. F., Preliminary studies on the development of a microbiological treatment for polychlorinated biphenyls. *Arch. Environ. Contam. Toxicol.*, **19** (1990) 789–96.
8. Smart, N. A., *Res. Rev.*, **64** (1976) 1–16. Cited by Horwitz, W. in *Adv. Pestic. Sci.*, Part 3 (1979) 649–55.
9. Katayama, A. & Matsumura, F., Degradation of organochlorine pesticides, particularly endosulfan, by *Trichoderma harzianum. Environ. Toxicol. Chem.*, **12** (1993) 1059–65.
10. Sahu, S. K., Patnaik, K. K., Sharmila, M. & Sethunathan, N., Degradation of alpha-, beta-, and gamma-hexachlorocyclohexane by a soil bacterium under aerobic conditions. *Appl. Environ. Microbiol.*, **56**(11) (1990) 3620–2.